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The Aer protein and the serine chemoreceptor Tsr independently sense intracellular energy levels and transduce oxygen, redox, and energy signals for *Escherichia coli* behavior

(signal transduction/bacterial chemotaxis/aerotaxis)

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ABSTRACT **We identified a protein, Aer, as a signal transducer that senses intracellular energy levels rather than the external environment and that transduces signals for aerotaxis (taxis to oxygen) and other energy-dependent behavioral responses in *Escherichia coli*. Domains in Aer are similar to the signaling domain in chemotaxis receptors and the putative oxygen-sensing domain of some transcriptional activators. A putative FAD-binding site in the N-terminal domain of Aer shares a consensus sequence with the NifL, Bat, and Wc-1 signal-transducing proteins that regulate gene expression in response to redox changes, oxygen, and blue light, respectively. A double mutant deficient in *aer* and *tsr*, which codes for the serine chemoreceptor, was negative for aerotaxis, redox taxis, and glycerol taxis, each of which requires the proton motive force and/or electron transport system for signaling. We propose that Aer and Tsr sense the proton motive force or cellular redox state and thereby integrate diverse signals that guide *E. coli* to environments where maximal energy is available for growth.**

In an environment where oxygen is being depleted by growing bacteria, aerotaxis quickly moves *Escherichia coli* and *Salmonella typhimurium* to a more favorable microenvironment before anoxia develops (1). Aerotaxis, the behavioral response to oxygen, requires a functional electron transport system (2). Oxygen stimulates electron transport through the electron transport system, increasing the proton motive force (2, 3). A postulated aerotaxis-transducing protein responds to the increase in electron transport/proton motive force and initiates a signal for the behavioral response to oxygen (3–6). Chemicals other than oxygen that stimulate electron transport also elicit an aerotaxis-like behavioral response in bacteria (5, 7, 8). The aerotaxis transducer may mediate other types of bacterial behavior, such as the newly described taxis to a preferred redox potential (9) and energy taxis to carbon sources, such as glycerol (10) or proline (11). Each of these behaviors involves modulation of the proton motive force and electron transport as the initial sensory transduction event (3).

The aerotaxis sensory transduction pathway includes three proteins that are common to the chemotaxis pathway: the CheA sensor kinase, CheY cognate response regulator, and CheW docking protein (12). The methyl-accepting chemotaxis receptors Tsr, Tar, Trg, and Tap (13–15) span the cytoplasmic membrane and have a highly conserved signaling sequence in the C-terminal cytoplasmic domain that binds CheA and CheW. Repellent binding to a chemotaxis receptor induces a conformational change in the signaling domain that increases the rate of CheA autophosphorylation. The phosphoryl residue from CheA is transferred to CheY, which, in its phosphorylated state, binds to a switch on the flagellar motors and signals a reversal of the direction of rotation of the flagella. Evidence that CheA, CheW, and CheY are also part of the aerotaxis response (12) led us to propose that the aerotaxis transducer would have (*i*) a C-terminal domain homologous to the chemoreceptor signaling domain that modulates CheA autophosphorylation and (*ii*) a domain that senses oxygen. We have identified a putative aerotaxis transducer in a computerized search of protein databases and confirmed that this protein, which we named "Aer," functions as a transducer for aerotaxis and related responses. We also present evidence that, in addition to Aer, the serine chemoreceptor Tsr functions as an independent transducer for aerotaxis and related responses. A preliminary report of these findings was presented at the Annual Meeting of the *American Society for Biochemistry and Molecular Biology*, 1996 (16).

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The strains and plasmids used are listed in Table 1. Cells were grown in Luria–Bertani medium containing 1 μM thiamine at 35°C to OD_{600} = 0.45–0.50, unless specified otherwise.

Construction of Mutants. A 1.2-kb kanamycin cassette excised from pMB2190 (a derivative of pUC4K obtained from P. Matsumura, University of Illinois, Chicago) using *Hin*cII was ligated into pGH1 at the *Sma*I site (nt 349) of *aer*. The *aer*-2::*kan* construct was excised with *Xmn*I and *Ehe*I and ligated into the *Sma*I site of the temperature-sensitive plasmid pKO3 (kindly provided by G. M. Church, Harvard University, Cambridge, MA). Mutants defective for *aer* were generated by allelic exchange as described by Hamilton *et al.* (23) and modified by Link (24). The pKO3 vector contains a *Bacillus subtilis sacB* gene that inhibits *Escherichia coli* growth in the presence of sucrose and facilitates the selection of cells that have lost the vector sequence. Gene replacement was verified by PCR using Expand Long *Taq* Polymerase (Boehringer Mannheim)(25) and Southern blot analysis of the transformants. The absence of fusion products was confirmed by sequencing the inactivated gene.

The *aer tsr* double mutant BT3311 was generated by P1 transduction of a *thr*$^+$ Δ*tsr*-7021 fragment from RP5882 into BT3309 (*aer*-2) recipient cells and by selecting for threonine$^+$ transductants. Colonies that lacked serine taxis on tryptone

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This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviation: IPTG, isopropylthiogalactoside.
A commentary on this article begins on page 10487.
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Table 1. Strains and plasmids used in this study

Strains/ plasmids	Relevant genotype	Source/reference
Strains		
MM335	wild type	(17)
RP437	wild type	(18)
GK100	Δ*cyo* Δ*cyd*	(19)
RP5882	Δ(*tsr*) *7021*	(20)
BT3300	MM335 *aer-2*::*kan*	This study
BT3309	RP437 *aer-2*::*kan*	This study
BT3310	BT3309/pGH1 (Aer^{++})	This study
BT3360	MM335/pGH1 (Aer^{++})	This study
BT3311	*aer-2*::*kan* Δ*tsr*	This study
BT3313	BT3311/pJL3 (Tsr^{++})	This study
Plasmids		
pK03	*rep* pSC101(*ts*) *sacB*	G. M. Church*
pTrc99A	p_{tac} *lacI*^{q}	Pharmacia and (21)
pGH1	pTrc99A *aer*$^+$	This study
pJL3	p_{tac} *lacI*^{q} *tsr*$^+$	(22)

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semisoft agar were further characterized by temporal chemotaxis assays.

Overexpression of Aer and Tsr. The expression vector pGH1 was constructed by subcloning a 1.5-kb *Afl*III–*Sal*I PCR fragment containing the *aer* gene into the *Nco*I and *Sal*I sites of pTrc99A and was verified by sequencing. pGH1 was introduced into BT3309 cells by electroporation and was induced with varying amounts of isopropylthiogalactoside (IPTG).

The plasmid pJL3 (22) containing the *lacI*^{q} repressor gene and the *tsr* gene under p_{tac} control was electroporated into BT3311. The transformants were selected on Luria–Bertani medium containing ampicillin (60 μg/ml) and grown in the presence of ampicillin, and Tsr overproduction was induced with 25 μM IPTG.

Behavioral Assays. Spatial and temporal gradient assays were used to assess aerotaxis, redox taxis, and taxis toward glycerol. For the spatial aerotaxis assay, cells were loaded into an optically flat capillary, and formation of an aerotactic band of bacteria near the air-liquid interface was observed and video-recorded using a dark-field video microscope (26). To quantitate the aerotactic response, a temporal assay for aerotaxis (2) was timed either by inspection or by computerized motion analysis, as described (26). Video images of free-swimming bacterial cells were digitized at 10 frames per second using a VP110 video processor (Motion Analysis, Santa Rosa, CA). Tumbling frequency was determined using a program based on EXPERTVISION software (27).

Chemotaxis to glycerol was determined on glycerol (1 mM) H1 minimal swarm plates and in a temporal assay (10, 28). Redox taxis was monitored as a repellent response to 2, 3-dimethoxy-5-methyl 1,4-benzoquinone in a temporal assay (9).

Measurement of Oxygen Concentration. Respiration rates of bacterial suspensions were measured using a Clark-type electrode and an oxygen monitor (Yellow Springs Instruments) that was connected to a MacLab MKIII data recording system (Analog Digital Instruments, Milford, MA).

Database Searches and Sequence Analysis. A search of combined protein databases was performed using a BLASTP program (29). The highly conserved domain of Tsr (residues 371–400) that is present in the C terminus of all chemotaxis receptors (30) was used as a query. Transmembrane regions were identified using the TMAP program (31). Multiple alignments of protein sequences were accomplished using the CLUSTAL W program (32).

RESULTS

Identification and Sequence Analysis of the *aer* Gene. An ORF (ORF506) at 69.1 min on the *E. coli* chromosome was sequenced by the Blatner group in the *E. coli* Genome Project and deposited in the GenBank database (accession no. U28379). The sequence currently is listed as AIR_ECOLI in the SwissProt database (accession no. P50466). Transcription of the ORF506 gene is independent of the flanking *ygjG* and *yqjI* genes. A computerized search revealed that the putative product of the ORF506 was a 506-residue protein that had a predicted C-terminal segment with 96.7% identity to a highly conserved domain of Tsr (Fig. 1*C*). The N-terminal domain of the ORF506 protein (residues 8–129) shares homology with domains in the NifL protein of *Azotobacter vinelandii* (28% identity, 52% similarity), the Bat protein of *Halobacterium salinarium* (22% identity, 54% similarity) and Wc-1, the White Collar protein of *Neurospora crassa* (19% identity, 37% similarity) (Fig. 1*A*). Both NifL and Bat are known oxygen sensors. In response to oxygen, the NifL protein regulates expression of nitrogen fixation genes (33), and the Bat protein regulates synthesis of bacteriorhodopsin (34). The Wc-1 protein is a central regulator of blue light responses in *Neurospora* (35). Thus, the ORF506 protein had the expected domains of the putative aerotaxis-transducing protein (12), and the gene was renamed "*aer*" (*a*erotaxis and *e*nergy *r*esponses) after experimental evidence confirmed its function.

Sequence analysis predicted that the topology of Aer in the membrane is different from the topology of known chemotaxis receptors that transverse the cytoplasmic membrane (TM1 and TM2) at either end of a periplasmic ligand-binding domain. Only one hydrophobic sequence (residues 167–206) was predicted in Aer. This sequence and these flanking regions are similar to the TM1 and TM2 transmembrane regions of Tsr (Fig. 1*B*) but lack the intervening periplasmic domain. Cytoplasmic sequences known to maintain the correct orientation of TM1 and TM2 in the membrane, namely three positively charged residues adjacent to TM1 and an amphipathic sequence adjacent to TM2 (36), are present in Aer (Fig. 1*B*). The similarity of the Aer hydrophobic sequence to TM1 and TM2 and the presence of a central proline residue (Pro-186) suggest that the hydrophobic sequence of Aer forms a tight hairpin of two transmembrane spans. This would place the N terminus of Aer in the cytoplasm, as it is in Tsr. The major difference between the two receptors is that the periplasmic sensing region of Tsr is missing in Aer, and the proposed cytoplasmic N-terminal region (residues 1–166) is a putative sensing domain in Aer but is truncated (residues 1–10) in Tsr.

Aer Is an Aerotaxis Signal Transducer. The possible role of the Aer protein in aerotaxis was investigated by constructing isogenic *E. coli* mutants in which the *aer* gene was inactivated by insertion of a kanamycin cassette through allelic exchange. The parent (RP437) and isogenic *aer-2*::*kan* mutant (BT3309) were examined for aerotaxis in a capillary assay (26). In this assay, an oxygen gradient is created by the bacteria when they consume oxygen that has diffused across the meniscus. The wild-type cells migrated to form sharply focused aerotactic bands within 10 min (Fig. 2*A*). The *aer* mutant formed a band that was further from the meniscus, indicating that the mutant cells were attracted to a lower oxygen concentration than the wild-type cells. The band formed by the *aer* mutant was also more diffuse.

To quantitate the aerotactic response, we used a temporal gradient assay that does not rely on cell respiration to form the oxygen gradient and that allows accurate determination of adaptation times in response to an increase or decrease in oxygen concentration (2). The temporal assays provided important details about the aerotaxis response that were not revealed by the spatial assays. The duration of the response of the *aer-2*::*kan* mutant to either an increase or a decrease in oxygen concentration was approximately half that of the parent (Fig. 2*B*; Table 2). No polar effects of the cassette insertions were expected because the *aer* gene is transcribed independently and is not located in an operon. To verify the

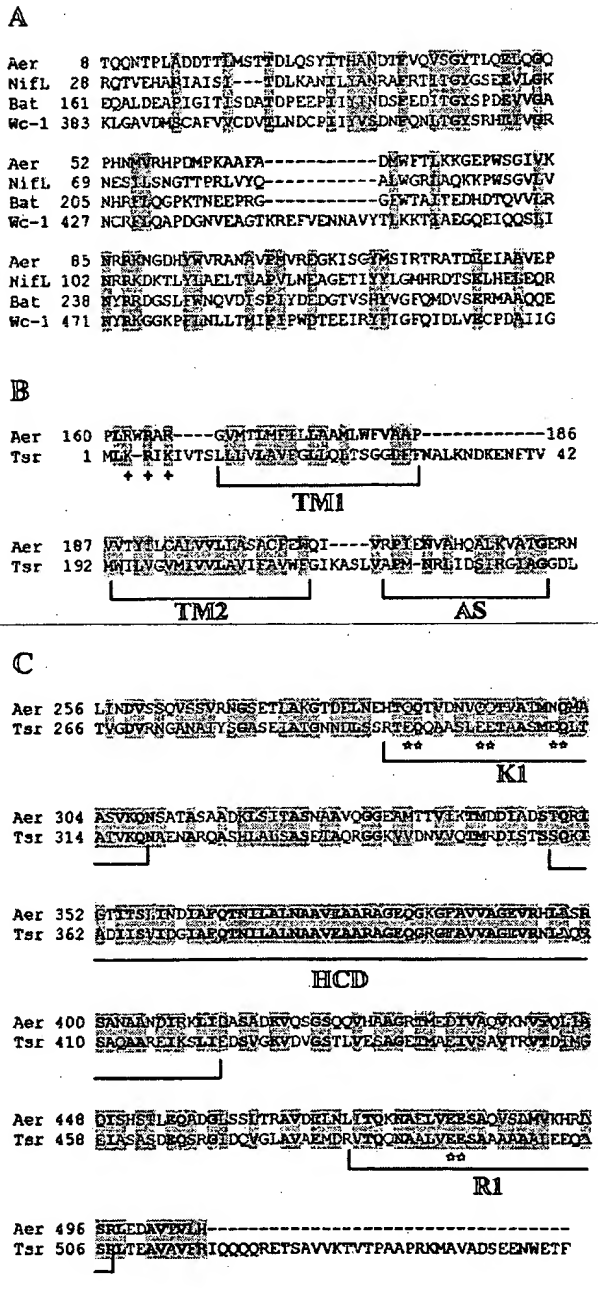

FIG. 1. The domain structure of the Aer protein in *E. coli* and comparison of its deduced amino acid sequence to homologous proteins. Similar residues are highlighted; identical residues are in bold. (*A*) Multiple alignment of the N-terminal domain of Aer with homologous domains of three related proteins, NifL from *A. vinelandii*, Bat from *H. salinarium*, and Wc-1 from *N. crassa*. (*B*) Alignment of the transmembrane region of Aer with the transmembrane regions (TM1 and TM2) of Tsr. Three positively charged residues (+) and the amphipathic sequence (AS) adjacent to the transmembrane regions of the Tsr protein are indicated. (*C*) Alignment of the C-terminal domains of Aer and Tsr. Methylation regions, K1 and R1, and the highly conserved domain (HCD) are shown. The Glx–Glx doublets corresponding to characterized methylation sites of Tsr (37) are marked with an asterisk. See text for details.

absence of polar effects, we tested a strain with an in-frame deletion of *aer* (*aer*-1, UU1117), obtained from Bibikov and Parkinson (38). The *aer*-1 strain showed both the impaired band formation in a capillary and decreased aerotaxis in a temporal assay that were observed with the *aer-2*::*kan* mutant (data not shown).

To confirm the involvement of Aer in signal transduction in aerotaxis, we quantitated the aerotactic responses of cells in which Aer was expressed from the tightly regulated plasmid, pGH1, introduced into the *aer-2*::*kan* mutant. Expression of Aer restored the wild-type aerotaxis phenotype, and the intensity of aerotaxis correlated with the level of expressed Aer. In a variety of strains that were tested, the tumbling response to an oxygen decrease (Fig. 3) and the smooth swimming response to an oxygen increase (data not shown) progressively lengthened when Aer was amplified by increasing amounts of IPTG.

Aer-dependent behavioral changes were not the result of a general effect on bacterial chemotaxis. For example, Aer overproduction did not increase the response to 10 μM serine, mediated by the Tsr receptor (BT3310, Table 2), or the response to 20 μM mannitol, a receptor-independent response mediated by the phosphotransferase system (39) (data not shown). Taken together, the results above are consistent with a role for Aer as a transducer for aerotaxis and also suggest that there is an additional aerotaxis transducer in *E. coli*. Bibikov *et al.* (38) independently identified Aer as a transducer for aerotaxis in *E. coli*.

The Serine Chemoreceptor, Tsr, Is a Second Transducer for Aerotaxis. We investigated the characteristics of aerotactic responses that are transduced by the unidentified second aerotaxis sensor. It was confirmed that there were no aerotaxis responses in a *cyo cyd* strain (GK100) that lacks the cytochrome *o* and cytochrome *d* terminal oxidases (19). This is evidence that electron transport to the terminal oxidase is required for both Aer-dependent aerotaxis and aerotaxis mediated by the unidentified aerotaxis sensor.

It has been shown that aerotaxis is inverted in the *cheB* mutant; addition of oxygen causes a repellent response, and removal of oxygen causes an attractant response (40). However, aerotaxis returns to normal if the *tsr* gene also is deleted in the *cheB* mutant (40). This suggested a possible role of Tsr in aerotaxis.

A Δ*tsr* mutant (RP5882) was examined for aerotaxis in spatial and temporal aerotaxis assays. The mutant cells formed the aerotactic band in a capillary at a higher oxygen concentration than wild-type cells (Fig. 2*A*) and were indistinguishable from wild-type cells in a temporal assay (Table 2). An *aer tsr* double mutant (BT3311) was constructed and examined for aerotaxis. Aerotaxis was abolished in the *aer tsr* double mutant. No aerotactic bands formed in a spatial oxygen gradient (Fig. 2*A*), and we observed no responses to an increase or decrease in oxygen concentration in a temporal assay (Table 2). The double mutant did respond, however, to both aspartate (Tar-dependent chemotaxis) and to mannitol (phosphotransferase chemotaxis) (data not shown). Aerotaxis was restored in the *aer tsr* double mutant when Tsr (Table 2) or Aer (data not shown) was expressed from a plasmid.

These results indicate that Tsr is a second transducer for aerotaxis. The two transducers function independently because Tsr is not essential for Aer-mediated aerotaxis and vice versa.

Aer and Tsr Transduce Redox and Energy Signals. It was previously postulated that an aerotaxis transducer may mediate other types of bacterial behavior that involve changes in electron transport and the proton motive force (3–6). To evaluate whether Aer and Tsr sense other stimuli that modulate electron transport and the proton motive force, we tested the responses of *E. coli* strains to glycerol and redox potential. Glycerol was recently demonstrated to elicit energy taxis in *E. coli* (10). The bacteria do not sense glycerol *per se* but apparently respond to the increased electron transport and proton motive force that results from glycerol metabolism. We recently described redox taxis in *E. coli*, a response that also involves sensing the proton motive force or the redox state of the electron transport system (9). Responses to glycerol and

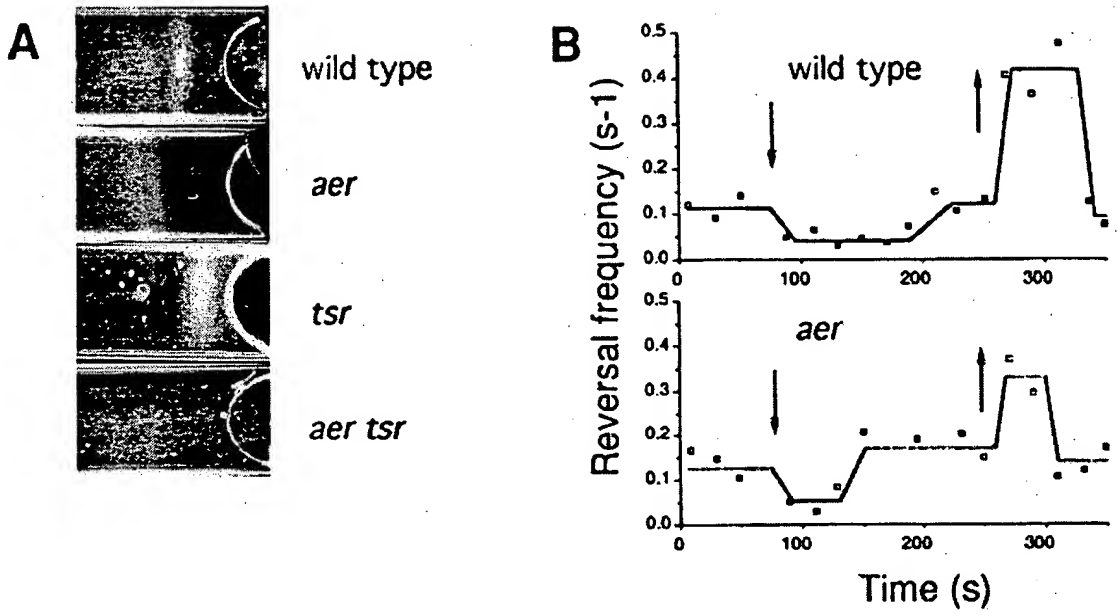

FIG. 2. Comparison of aerotactic behavioral responses in wild-type and mutant strains of *E. coli*. (*A*) Aerotaxis of *E. coli* RP437 and isogenic mutants, BT3309 (*aer*), RP5882 (*tsr*), and BT3311 (*aer, tsr*) in a spatial oxygen gradient. A capillary assay was performed as described in *Materials and Methods*. Photographs were taken 10 min after the cells were placed into capillaries. The meniscus at the air interface is visible at the right in the photographs. (*B*) Aerotactic response in an *aer* mutant (BT3300) and its parent (MM335) in a temporal gradient assay using computerized motion analysis as described in *Materials and Methods*. Arrows indicate addition (↓) and removal (↑) of oxygen. The MM335 strain shows enhanced aerotaxis and chemotaxis responses compared with RP437.

redox effectors in strains deficient in *aer* and *tsr* were tested using a temporal gradient assay (Table 2). No taxis to 1 mM glycerol or to the redox repellent 2, 3-dimethoxy-5 methyl-1,4 benzoquinone (10 μM) were observed in the *aer tsr* double mutant (BT3311). Overproduction of Aer (BT3310) significantly enhanced both glycerol taxis and redox taxis (Table 2). We conclude that Aer and Tsr are transducers for sensing of redox potential and energy status, in addition to sensing oxygen.

DISCUSSION

Aer and Tsr as Signal Transducers for Energy-Dependent Behavior. This study identified two independent signal transducers for energy-dependent behavioral responses in *E. coli*. A protein, Aer, sensed changes in the rate of electron transport/proton motive force that resulted from addition (or removal) of oxygen or a carbon source and from interaction of the electron transport system with artificial electron carriers, such as quinone analogs. Overproduction of Aer increased the response time to both positive and negative stimuli that affect the electron transport system. Insertional inactivation of the *aer* gene decreased the response time to those stimuli and caused changes in bacterial behavior in a spatial gradient of oxygen. The energy-dependent behavioral responses were not eliminated by the *aer* insertional inactivation or deletion, indicating that there is a second transducer for aerotaxis and related responses. We identified the serine chemoreceptor, Tsr, as the second transducing protein. The *aer tsr* double mutant lacked all of the energy-related responses but had normal chemotaxis to aspartate and phosphotransferase sugars. The energy-dependent responses (as well as chemotaxis to serine) were restored when the *tsr* gene was expressed from a plasmid in the *aer tsr* double mutant, clearly confirming the role of Tsr as a second transducer. Although not observed in this study, we have not completely eliminated the possibility that other known chemotaxis receptors of *E. coli*, Tar, Trg, and Tap, can transduce signals for energy-dependent behavior. In an analogous system, Tsr was initially considered to be the sole receptor for thermosensing in *E. coli* (41). Subsequently, in a *tsr* strain, the Tar protein was shown to be a transducer for the residual thermotaxis (42), and Trg and Tap were transducers

Table 2. The behavioral responses of wild-type and mutant strains of *E. coli* in temporal gradients of oxygen, a quinone analog, glycerol, and serine

Strain	Relevant genotype	Behavioral response to stimulus and time for adaptation, s				
		O_2 increase, 0–21%	O_2 decrease, 21–0%	Glycerol, 1 mM	Quinone,* 10 μM	Serine, 10 μM
RP437	wild type	51 ± 9 (smooth)	30 ± 3 (tumbly)	74 ± 21 (smooth)	133 ± 12 (tumbly)	113 ± 14 (smooth)
BT3309	*aer*	30 ± 3 (smooth)	15 ± 6 (tumbly)	44 ± 8 (smooth)	96 ± 7 (tumbly)	119 ± 10 (smooth)
BT3310	*aer* pGH1 (Aer^{++})†	>300 (smooth)	76 ± 3 (tumbly)	>180 (smooth)	>300 (tumbly)	100 ± 3 (smooth)
RP5882	*tsr*	55 ± 9 (smooth)	32 ± 3 (tumbly)	52 ± 2 (smooth)	185 ± 48 (tumbly)	NR
BT3311	*aer tsr*	NR	NR	NR	10 ± 3 (tumbly)	NR
BT3313	*aer tsr* pJL3 (Tsr^{++})‡	>180 (smooth)	29 ± 18 (tumbly)	>180 (smooth)	>180 (tumbly)	>180 (smooth)

Response time was measured as the time interval required fot 50% of the bacteria to return to prestimulus behavior. The results shown are the mean ± SD calculated from two independent experiments with three replicates in each.
*2,3 dimethoxy-5-methyl 1,4 benzoquinone.
†Aer overproduction was induced with 100 μM IPTG.
‡Tsr overproduction was induced with 25 μM IPTG.
NR, no response.

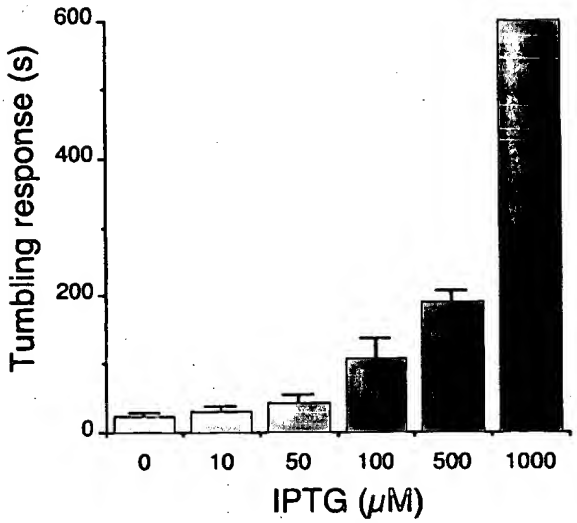

FIG. 3. The response of *E. coli* to an oxygen decrease as a function of expression of *aer*. Wild-type MM335 cells transformed with pGH1 were grown to mid-log phase (OD_{600} = 0.4–0.5) and induced with the indicated concentrations of IPTG for 1 h before analysis of aerotaxis by a temporal assay. In the presence of 1 mM IPTG, the cells did not adapt in the 600 s that they were observed.

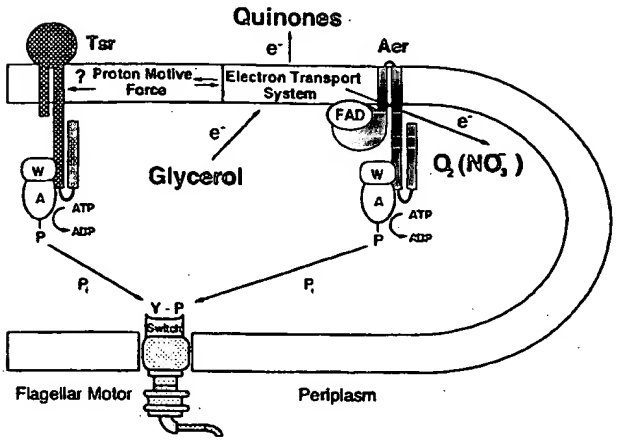

FIG. 4. Model for energy-sensing by Aer and Tsr. Aer senses modulators of the electron transport system. This is postulated to be mediated by the FAD cofactor of Aer. Tsr may sense the proton motive force directly or indirectly through changes in the electron transport system. Homologous signaling domains in Aer and Tsr bind to the CheA/CheW complex and ultimately regulate the level of phosphorylation of the CheY response regulator. A, CheA sensor kinase; W, CheW docking protein; Y, CheY response regulator; Z, CheZ phosphatase.

when overexpressed in a strain that was negative for chemotaxis receptors (43). This suggests that thermotaxis transduction signals originate from one of the conserved domains of the receptors.

A Signal Transduction Model for Aerotaxis and Related Responses. Previous studies of the behavioral responses of *E. coli* to oxygen (2, 44), other terminal electron acceptors (5), redox molecules (electron carriers) (9), and rapidly metabolized carbon sources (electron donors) (10) demonstrated that a signal for each of these behaviors is generated in the electron transport system. Electron transport and proton motive force are tightly coupled, so it is not possible at this time to distinguish which is the signal for bacterial behavior. In this study, "energy-sensing" and "redox-sensing" are used interchangeably to refer to transducers that sense changes in the proton motive force/electron transport system. Environmental changes that result in an increase in the cellular energy level cause positive taxis. For example, an increase in electron donors and acceptors and a decrease in the concentration of de-energizing redox molecules would be interpreted as positive stimuli. Changes that result in a decrease in the energy level cause negative taxis. We propose that the term "energy taxis" be used for all such behaviors. This term reflects the biological significance of the behaviors': navigation of bacteria to niches where the combination of environmental factors is optimal for energy generation and therefore for growth and homeostasis.

A model for energy sensing (Fig. 4) incorporates the findings of this study. Any chemical that alters the flux through the electron transport system will induce an equivalent change in the proton motive force. The change in redox/energy level is transduced by Aer and Tsr into energy-taxis signals that alter the rotational bias of the flagellar motors.

The consensus sequence in the Aer, NifL, Bat, and Wc-1 proteins (Fig. 1*A*) is a putative FAD-binding site, recently identified in NifL (33). Bibikov *et al.* (38) detected an increased amount of FAD in *E. coli* membranes upon overproduction of the Aer protein. This observation is consistent with the hypothesis that Aer is an FAD-containing flavoprotein. In preliminary experiments, we have isolated and purified the Aer protein and found a noncovalently bound cofactor that comigrates with FAD in the HPLC profiles (unpublished data). The midpoint redox potential of FAD/$FADH_2$ is −220 mV; the redox potential of Aer-bound FAD is likely to be in the range that permits interaction with dehydrogenases (−300 mV range) and/or with ubiquinone (+113 mV) and menaquinone (−74 mV) (9, 44, 45). As a result, Aer may sense changes in the electron transport between dehydrogenases and quinones. Aer could also sense indirectly changes in the proton motive force because a proton motive force-dependent reversal of electron transport could reduce FAD.

Tsr has no redox prosthetic group, and it is possible that Tsr directly senses the proton motive force. Tsr has cytoplasmic and periplasmic residues that are proposed to be involved in pH-sensing (46). Proteins may also be capable of sensing the electrical component ($\Delta\Psi$) of the proton motive force (47). However, it remains to be determined whether Tsr senses electron transport or the proton motive force. Tsr is well known as a protein with many roles, including sensing of the external (serine, leucine) and internal (pH) environments (48). The present study adds energy-sensing to the list of known roles of Tsr.

The existence of a proton motive force sensor in bacteria has long been proposed (5, 49). Previous attempts to identify the proton motive force sensor have been unsuccessful, but this study identifies both Aer and Tsr as direct or indirect sensors of the proton motive force. As such, Aer is a sensor of the internal and not the external environment of the cell. This may be an alarm response that alerts the cell in an environment that threatens the maintenance of optimal energy levels in the cell. A proton motive force sensor may be widespread in living systems. A consensus sequence shared by the Aer, NifL, Bat, and Wc-1 sensory proteins is shown in Fig. 1*A*. Further studies have identified a highly conserved PAS domain in Aer that is present in diverse sensory proteins from the Archaea to humans (50).

The mechanism of adaptation in aerotaxis is not clear. Aer has three putative methylation sites in its C terminus (two in the K1 region and one in the R1 region) (Fig. 1*C*); however, significant deviations from the consensus sequence observed in the chemotaxis receptors (30) raise a question as to whether Aer can be methylated by the CheR methyltransferase. Aerotaxis can occur in the absence of protein methylation (39, 40). Adaptation in Tsr-mediated aerotaxis is likely to be methylation-dependent. We have recently demonstrated methylation-

dependent adaptation of aerotaxis in *B. subtilis* (51) and *H. salinarium* (27).

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